458

THE ROLE OF GAP JUNCTION FOR INFLAMMATORY CYTOKINES IN FIBROBLAST-LIKE SYNOVIOCYTES OF JOINT DISEASE

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Purpose: To investigate whether connexin43 (Cx43) gene silencing in fibroblast-like synoviocytes (FLSs) of rat in vitro by RNA interference enhances inflammatory cytokines such as tumor necrosis factor (TNF)-α, interleukin (IL)-1β, and IL-6.
Methods: Small interfering RNA (siRNA) duplexes targeting the rat Cx43 gene were synthesized. In order to investigate whether interference of Cx43 gene expression affect the inflammatory cytokine, the cells were transfected with 50nM of siRNA 48hr before stimulation with lipopolysaccalide (LPS/0.1 μg/ml). After 6hr stimulation with LPS, total RNAs were extracted from the cells. The expression levels of Cx43, TNF-α, IL-1β, and IL-6 mRNA were analyzed by quantitative real-time PCR.
Results: After stimulation with LPS to FLSs, the expression of Cx43, TNF-α, IL-1β, and IL-6 mRNA were stimulated, respectively, compared to the cells without LPS (Fig 1). When transfection with Cx43 siRNA was performed, Cx43, TNF-α, IL-1β, and IL-6 expression were markedly reduced to 25.7±1.2, 56.1±9.3, 40.8±8.7, and 18.5±8.2 (%), respectively, compared to the cells which were transfected of non specific siRNA (Fig. 1).

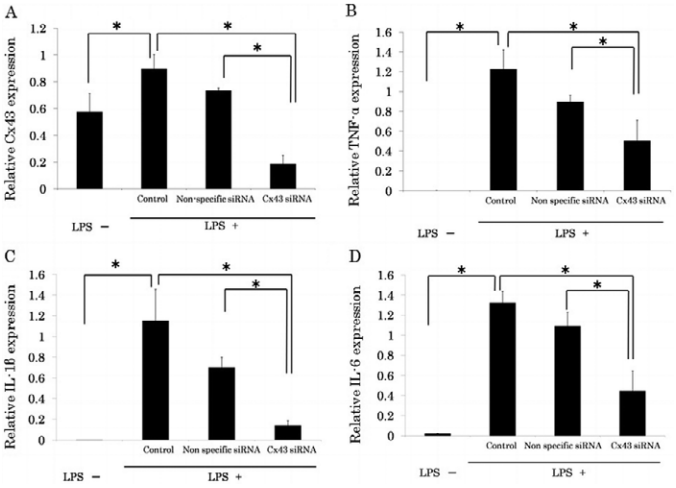

Figure 1. The expression of Cx43. TNF-α, IL-1β, and IL-6 mRNA after stimulation with LPS were stimulated. The mRNA levels of those with Cx43 siRNA treatment were markedly decreases (*P<0.01).

Conclusions: Gap junctions are specialized connections between cells in a tissue, and each gap junction consists of a number of individual hexagonal channels, each formed by 6 molecules of a structural protein known as connexin. Several recent studies have shown that intercellular communication facilitated by gap junctions may play an important role in the early development of osteoarthritis (OA). Marino, et al reported that synovial tissue from patients with OA has been found to have 4 times the number of gap junctions as that from unaffected patients, and also showed that the level of Cx43 which was the main protein in gap junction protein was 50% higher in osteoarthritis synovial tissue than in control synovium. In the pathogenesis of OA, various inflammatory cytokines produced by synovium leads to destruction of joints. In particularly, proinflammatory cytokines such as TNF-α, IL-1β, and IL-6 are known as important mediators in OA. Therefore, to suppress the expression of TNF-α, IL-1β, and IL-6 in joints in vivo is expected an effective and less invasive conservative therapy of joint disease such as OA. In this study, we elucidated that the Cx43 mRNA levels highly expressed into FLSs by stimulation of LPS, and that the expression of all of those genes in Cx43 siRNA transfected FLSs was down-regulated. These results suggest that targeted down-regulation of Cx43 gene by siRNA may have the potential of the treatment for inflammatory joints.

459

INHIBITION OF CYTOKINE INDUCED PROSTAGLANDIN E2 PRODUCTION AND NF-κB ACTIVATION IN ARTICULAR CHONDROCYTES BY AVOCADO/SOYBEAN UNSAPONIFIABLES AND GLUCOSAMINE

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Purpose: We determined whether the combination of avocado soybean unsaponifiables (ASU) and glucosamine (GLU) is more effective in suppressing cytokine induced production of pro-inflammatory mediators than either ASU or GLU alone. Pro-inflammatory mediators such as prostaglandins, cytokines, chemokines, and nitric oxide are produced in excess in osteoarthritis (OA). They participate in the destruction of articular cartilage by inducing enzymes that degrade extracellular matrix. PGE_2 is one of the key prostaglandins that can activate enzymes capable of breaking down cartilage. PGE_2 also sensitizes nociceptors in peripheral nerve endings resulting in inflammatory pain. Production of PGE_2 is regulated by cyclooxygenase-2 (COX-2) which is controlled by the NF-κB transcription factor. NF-κB plays a critical role in the control of cell signaling in the inflammation pathway. Studies have demonstrated that ASU and GLU individually inhibit production of inflammatory mediators. Each compound has been reported to reduce inflammation and relieve pain in OA patients with no adverse effects. Individually, ASU and GLU are documented to be chondroprotective with structure modifying effects on osteoarthritic knees.
Methods: Equine chondrocyte cultures were verified for continued expression of the cartilage phenotype markers type II collagen and aggrecan by immunostaining and Western blot. Chondrocytes were seeded onto 6-well plates overnight to evaluate their response to test compounds. They were pre-incubated with: control media alone, ASU (NMX1000®-ASU; 10 or 20 μg/ml) alone; GLU (5 or 10 μg/ml) alone, or the combination of different concentrations of ASU and GLU for 24 hrs. Chondrocytes were activated with IL-1β (10 ng/ml) for 1 hr to determine NF-κB activation by immunostaining, or for 24 hrs for PGE_2 analysis by ELISA. Data was analyzed by one-way ANOVA, Tukey post-hoc, $p<0.05$ level of significance.
Results: Chondrocytes continued to synthesize type II collagen and aggrecan with negligible amounts of type I collagen, verifying phenotype retention. Chondrocytes cultured for 24 hours with control media alone produced low levels of PGE_2. Activation with IL-1β significantly increased PGE_2 production approximately 10-100 fold ($p<0.001$). Increased PGE_2 production was paralleled by translocation of NF-κB from the cytoplasm to the nucleus indicating activation. ASU alone or GLU alone marginally suppressed PGE_2 production. The combination of ASU and GLU resulted in at least 50% reduction in PGE_2 synthesis, which is significantly lower than ASU ($p<0.001$) or CS alone ($p<0.001$). The ASU and GLU combination also significantly suppressed NF-κB translocation from the cytoplasm to the nucleus by nearly 50%.
Conclusions: The present study shows that the combination of ASU and GLU inhibited cytokine induced PGE_2 production more effectively than either ASU or GLU alone. Inhibition of PGE_2 production is associated with suppression of NF-κB activation. Since PGE_2 production is dependent on COX-2, it is likely that the effect of ASU and GLU is mediated at least in part via NF-kB dependent inhibition of COX-2 expression. It is also possible that their modes of action involves different signaling pathways. Our observations suggest that ASU and GLU in combination may offer an effective anti-inflammatory and chondroprotective approach for the management of OA.

460

DIFFERENTIAL EFFECT OF HYALURONAN MOLECULAR WEIGHT ON INFLAMMATORY RESPONSE INDUCED BY LIPOPOLYSACCHARIDE IN HUMAN FIBROBLASTS

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Purpose: Osteoarthritis is one of the most common inflammatory diseases, affecting about 10% of the overall world population and involving the loss of joint functionality. The restoration of the normal function and pain relief of the affected joints are the main objectives of viscosupplementation, that is, hyaluronan (HA) intraarticular injection. HA is a glycosaminoglycan